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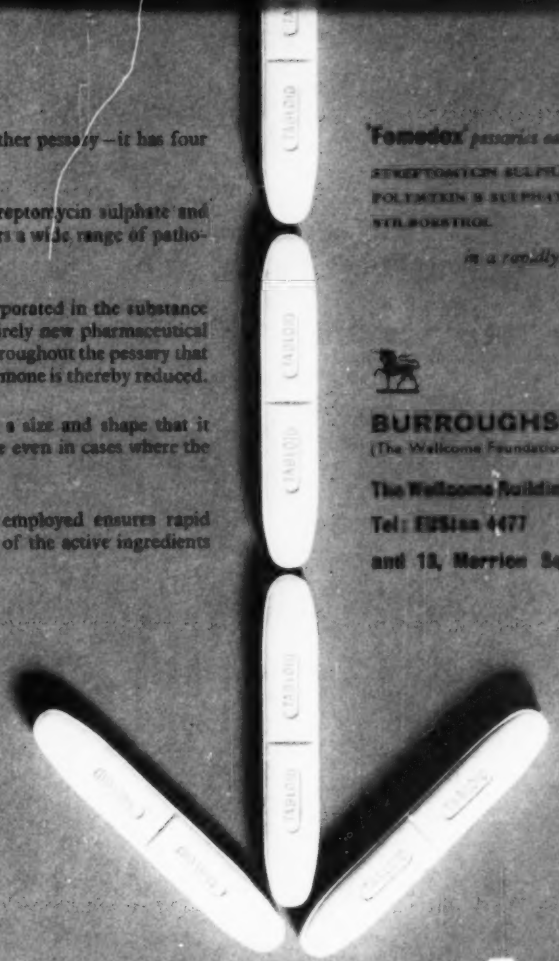

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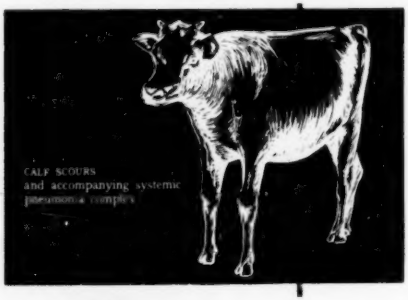

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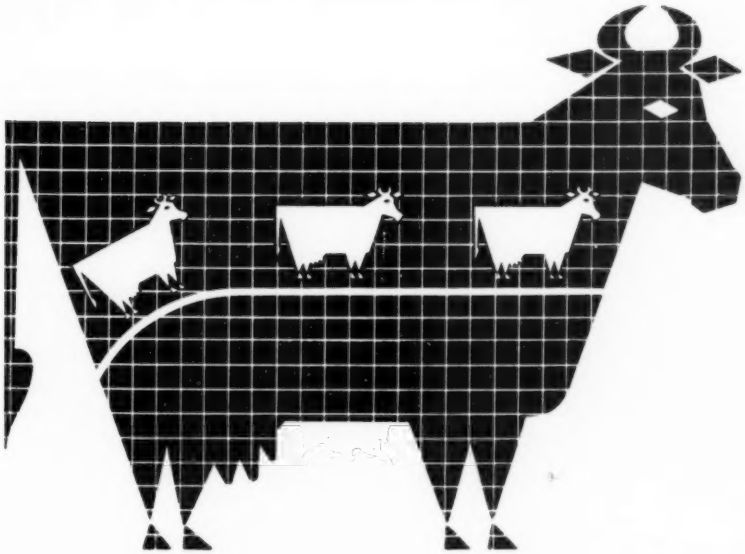

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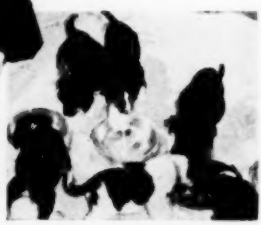

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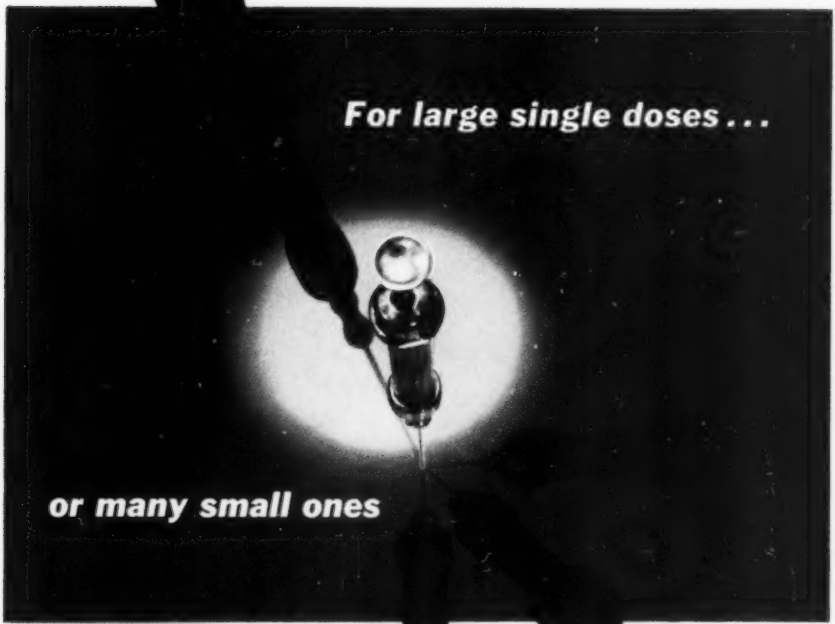

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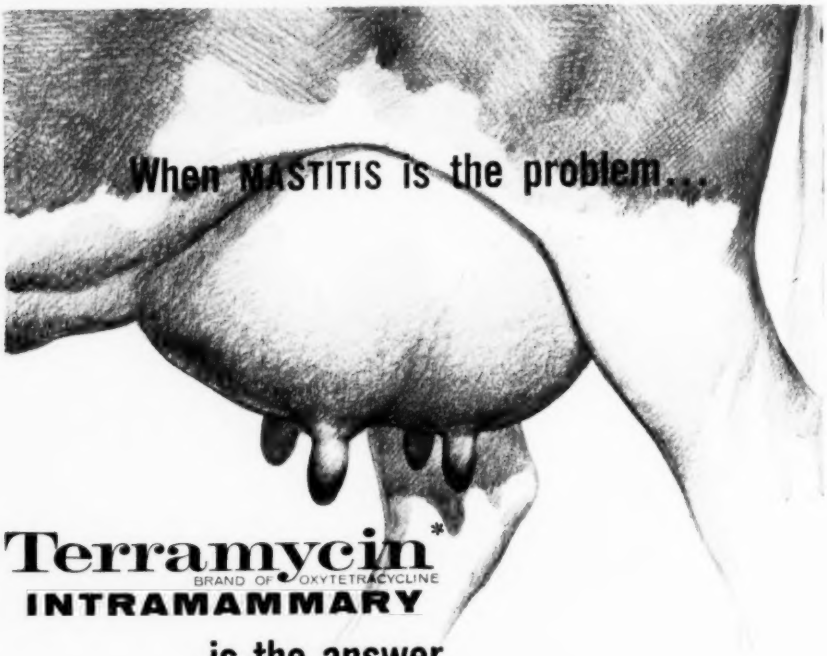

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CHRONIC COPPER POISONING IN SHEEP FOLLOWING THE USE OF COPPER SULPHATE AS A MOLLUSCICIDE

J. F. Gracey and J. R. Todd

Veterinary Research Division, Ministry of Agriculture for Northern Ireland, The Farm, Stormont, Belfast.

Allcroft (1960) has recently drawn attention to the increase in the incidence of chronic copper poisoning over the past few years especially in sheep and cattle, and our records confirm that this is also the case in Northern Ireland. The majority of deaths arise as a result of the ingestion of excessive amounts of copper sulphate incorporated directly in feeding stuffs, but we wish to record an outbreak which is unusual in that it followed the use of copper sulphate as a molluscicide spray on grassland for control of liver fluke.

Pryor (1959) records the occurrence of chronic copper poisoning in sheep, following top-dressing with superphosphate containing copper sulphate, at a rate supplying 7 lbs. copper sulphate per acre. No heavy falls of rain occurred for some time, and sheep which were put on the area two days after top-dressing began to succumb after about six weeks. Four months after applying the copper sulphate, the copper content of the pastures was still markedly raised (up to 280 p.p.m. Cu in the dry matter). The outbreak described below is essentially similar.

HISTORY

In September 1959 three fields totalling about 40 acres were sprayed with a 1 per cent copper sulphate solution at the rate of 50 gallons per acre (i.e. 5 lbs. copper sulphate per acre). Very little rain fell during the next few weeks and slight scorching of the pasture occurred in places. Three weeks after spraying, a flock of 95 ewes was put on to graze this area plus about 15 acres of untreated pasture, and remained there continuously.

After about one month two ewes died, and from the symptoms of jaundice and haematuria a tentative diagnosis of chronic copper poisoning was made and confirmed by *post-mortem* examination and chemical analysis of liver and kidney. The flock was immediately withdrawn from the treated area, confined to untreated pasture and given supplements of hay and concentrates. In spite of removal from the source of copper four other ewes died, the last death occurring on January 17, 1960. As well as these ewes which died one other ewe showed typical symptoms of the haemolytic crisis, but after a period of about one week

of severe depression and inappetence the jaundice and haematuria abated and the ewe recovered without any treatment. Marston (1950) refers to Merino sheep as being able to survive one or more haemolytic crises of chronic copper poisoning, but it is apparently rare for a sheep of a British breed to do so.

Lambing commenced towards the end of February and proceeded uneventfully, there being no further deaths from copper poisoning. The lambing rate (130 per cent) was lower than normal but as there was not an abnormal number of stillbirths it does not seem likely that the high copper intake had any effect on the foetuses.

POST-MORTEM FINDINGS

The main *post-mortem* finding was that of generalised icterus. On section, the cut surfaces of the livers were from yellow to orange in colour, and the kidneys congested and black. In two cases the spleen was very enlarged and congested and the bladder contained dark-coloured urine.

BLOOD AND TISSUE COPPER LEVELS

Analysis of blood samples showed no elevation of blood copper levels other than in animals exhibiting clinical symptoms. Thirteen ewes and one ram—all clinically normal—had blood copper levels ranging from 80 to 130 μg. per 100 ml., while a sick ewe, which died two days later, had a blood level of 660 μg. Cu per 100 ml. It must be assumed that all the ewes in the flock had high levels of copper stored in their livers at this stage (confirmed by liver biopsy samples mentioned below), so that these results are consistent with earlier findings (see Garner, 1957) that the blood copper level rises rapidly shortly before the appearance of symptoms.

Liver copper levels in the ewes which died ranged from 1,300 to more than 2,600 μg. Cu per g. dry matter. Analysis of liver biopsy samples from two ewes showed the copper concentrations set out in Table I, and confirmed that the excretion of copper from the liver is very slow, the rate of reduction of the liver copper level averaging about 200 p.p.m. per month in each case. These ewes were grazing normal pasture at this time, but were not treated in any way. Both of them lambed normally during this period, one producing twins.

TABLE I.—COPPER CONCENTRATIONS IN LIVERS OF TWO SHEEP
(in μg. per g. dry matter)

Date	*Sheep* 1	*Sheep* 2
January 22nd	1,630	1,395
April 7th	742	—
June 25th	630	490

Foetal liver from one of the casualties did not contain an abnormal amount of copper, the level being 75 μg. per g. dry matter as compared to 2,600 μg. per g. in the liver of the mother. Kidney copper levels ranged from 100 μg. to 500 μg. per g. dry matter, and one sample of pericardial fluid contained 1,490 μg. copper per 100 ml.

A CLINICAL TRIAL OF DIMERCAPTOPROPANOL TO REDUCE LIVER COPPER LEVELS

McDonald (1946) reported increased excretion of copper by sheep after injection of dimercaptopropanol (B.A.L.) intravenously, though Cunningham (1950) found that it was not effective in reducing liver copper levels. In order to investigate the effect of B.A.L. on these ewes which had been exposed to a high copper intake, a clinical trial was started on December 16. Seventy-eight ewes were treated with 0·1 g. B.A.L. in arachis oil by intramuscular injection and fifteen were left untreated as controls. This treatment was repeated four times at two to three-day intervals and all ewes were examined for signs of jaundice, etc., thereafter.

In January two of the treated ewes died, one of them apparently not from a haemolytic crisis, the other, however, being a typical case. The livers of these two ewes contained 2,300 and 2,500 μg. copper per g. dry matter, so that it would appear that B.A.L. in the quantities given was effective neither in reducing liver copper levels nor in preventing the crisis. In addition the injection caused a temporary lameness in some of the animals.

In the control group only one ewe showed evidence of icterus, but, as mentioned above, recovered without treatment.

COPPER CONTENT OF HERBAGE

The copper content of samples of herbage taken from the sprayed area ranged from 60 p.p.m. to 220 p.p.m. in the dry matter during December, January and February (i.e. up to five months after spraying). In March levels of 40 p.p.m. to 80 p.p.m. were recorded, and samples of the new season's growth of grass taken in May contained 15 p.p.m. to 20 p.p.m. Cu in the dry matter. The corresponding figures for unsprayed pasture during this whole period were in the 10 to 20 p.p.m. range.

DISCUSSION

The circumstances of this outbreak of copper poisoning were similar to those described by Pryor (1959) in that the source of the excess copper was pasture, and the most interesting feature was the high copper content of the herbage up to five months after the application of the spray. The retention of copper by the grass was most probably due to the exceptionally dry autumn of 1959 which allowed it to be fixed or absorbed by the leaf. Subsequent rainfall during the winter months was very heavy, but this failed to remove the copper from the herbage. In fact, a sample taken from an area which had been flooded for two days was found to contain 190 p.p.m. Cu in the dry matter.

The failure of B.A.L. as a means of hastening excretion of copper accumulated in the liver is in agreement with the experience of Cunningham (1950) and although McDonald (1946) found increased urinary excretion after B.A.L. treatment, the total amounts of copper excreted were of the order of half a milligram—which is insignificant in relation to the total copper content of a liver.

SUMMARY

An outbreak of chronic copper poisoning is described, in which six out of 95 ewes died after grazing pasture which had previously been sprayed with a 1 per cent copper sulphate solution as a molluscicide in liver fluke control.

Copper contents of herbage up to 200 p.p.m. in the dry matter were recorded and these high levels persisted throughout the dormant winter period (up to five months after spraying) in spite of heavy rain and, in some cases, flooding.

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(*Received for publication*, 17 *August*, 1960)

ON THE CONSTANCY OF CREATININE EXCRETION IN THE URINE OF THE DAIRY COW

By Th. de Groot and J. H. Aafjes

Agricultural Bureau of the Netherlands Nitrogenous Fertilisers Industry, and Laboratory for Animal Physiology, Agricultural University College, Wageningen, Holland

In veterinary practice, where the collection of 24-hour samples of urine is very difficult, the quantitative chemical examination of the urine provides little information of real value when one has no measure of its concentration. This is true because the quantity of urine produced per unit of time is very variable, depending on the quantity and time of ingestion of water. For that reason a low concentration of a certain constituent in the urine does not prove that the animal excretes little of this substance, nor does a high concentration in a single sample mean that considerable quantities of this constituent are being continuously excreted.

As will be shown later the specific gravity proved to be inadequate as a measure of the concentration. A more reliable criterion was therefore sought. It is known that in man and animals, other than ruminants, the amount of creatinine excreted per unit of time is practically constant for any individual and depends chiefly on the quantity of muscle in the body (Brody, Proctor and Ashworth, 1934; Borsook and Dubnoff, 1947).

Although the creatinine content of the urine is often used in medical practice and in experimental work with laboratory animals (Hunter, 1928) it is rarely used in veterinary practice or in investigations with ruminants. A study of the literature leads to the conclusion that no proof has yet been given of the constancy of creatinine excretion in the ruminant. This is remarkable, especially when one considers that 25 years ago Brouwer (1935) stated: "the examination of the urine (of the ruminant) seems to me very promising, when at the same time the creatinine content is determined."

Dinning, Gallup & Briggs (1949), experimenting with steers on a uniform feed intake, found that the creatinine excretion in 24 hours remained constant in individual animals, in contrast to the creatine excretion which varied quite considerably from day to day. However, this investigation gives no information about the constancy of the creatinine excretion during the day. The same is true for the work of Blaxter & Wood (1951, *a*, *b*) in experiments with calves and of Orr (1918), Brody, Proctor & Ashworth (1934), Ashworth & Brody

(1933), Morris & Ray (1939) and Lindsay (1912) using dairy cows and other adult ruminants. Butcher & Harris (1957) state that they found the creatinine excretion to be independent of the protein ingestion in ruminants.

What is required is a reliable measure of the concentration of any sample of urine. As the ruminant occupies a rather special position, particularly with respect to digestion, it does not appear to be valid to apply to ruminants observations made with other species. Furthermore, as ruminants in contrast to other species have usually a rather high content of creatine in the urine, the constancy of the creatinine excretion does not seem to be at all certain. Harding & Gaebler (1922) stated that if, in addition to creatinine, creatine is also excreted, which is normal in children, it is not the quantity of creatinine excreted which is constant but the sum of creatinine and creatine. For these reasons it seemed worth while to determine the creatinine and creatine excretion in the course of 24 hours and on consecutive days.

METHODS

For the collection of the urine two methods have been used.

(1) A catheter was placed in position in the bladder so that urine could be collected continuously and its rate of production measured. The difficulty with this method was the irregular flow of the urine, in some periods no urine being collected. In these cases by pulling on the catheter urine could be collected, but sometimes the urethra was damaged in this way and blood appeared in the urine.

This method was also used in a cow on pasture, modified in so far as a pinchcock was put on the catheter, which was opened every hour. In this case also sometimes no urine was obtained spontaneously and finally blood appeared in the urine.

In view of the inconsistent results and danger of infection using the permanent catheter, it was decided to try an alternative method.

(2) The apparatus described by van Es & Vogt (1959) was used for the collection. By means of a wide-bore tube the urine was led into a vessel placed on a seesaw. This when moved down by the weight of the urine operated an electric contact and produced a time record of the passing of urine on a recorder ("Electrofact"). A small hole in the bottom of the vessel permitted urine to flow into another flask. The flow of current was interrupted again when the vessel returned to its original position (Fig. 1).

The specific gravity and the volume of the urine collected were measured and a sample taken for determination of creatinine by the method of Folin (1914) and creatine plus creatinine by Benedict's (1914) method. The creatine content was expressed as mg. per cent creatinine.

As the time that elapsed between collections of urine was known it was possible to calculate the creatinine and creatine production per minute.

Mercuric iodide was added to the urine as a preservative and the samples were stored in a refrigerator if the analysis was not immediately carried out. HgI does not interfere with the methods of estimation.

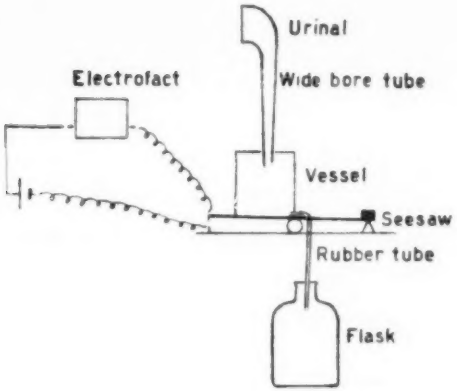

FIG. 1

RESULTS

A dry cow (Aly) of 550 kg. was kept on a ration of hay and water. On June 24, 1959 at 8 a.m. this animal was given 10 l. of water and at 9 a.m. a further 75 l. Then the catheter was introduced. At 10 a.m. the collection of urine was commenced and continued for 24 hours. In all, 20 samples were obtained. The volume of urine collected varied from 630–2,495 ml. per hour and the specific gravity from 1·007–1·018. The creatinine concentration ranged from 22–78 mg. per cent (Fig. 2) and the concentration of creatine plus creatinine from 36–119 mg. per cent.

The average creatinine excretion per minute was 9·47 ±0·48 mg. (s. = 2·14 mg.) (Fig. 2), the average creatine excretion per minute 6·11 ±0·33 mg. (s. = 1·46 mg.) and of creatine plus creatinine 15·60 ±0·85 mg. (s. = 3·82 mg.).

Between 9 and 10 p.m. only 620 ml. of urine were collected but in the following hour 2,030 ml. were obtained. It therefore seems likely that at 10 p.m. the bladder still contained a certain quantity of urine, which was voided during the next hour. For this reason it does not appear possible to obtain complete collection in every period and consequently entirely reliable figures for creatinine production.

Nevertheless in 10 out of 20 collection periods it was found that the creatinine excretion was between 9 and 10 mg. per minute, an indication of a constant creatinine output.

In Fig. 2 it will be seen that an apparent higher excretion always immediately followed a period of lower excretion, the excretion in excess of the mean being approximately equal to the deficit in the preceding period.

The constancy of creatinine excretion is more apparent when the large differences in the concentration of the various samples are considered. These variations were mainly caused by the fact that the cow drank 85 l. of water just before collection of urine was begun.

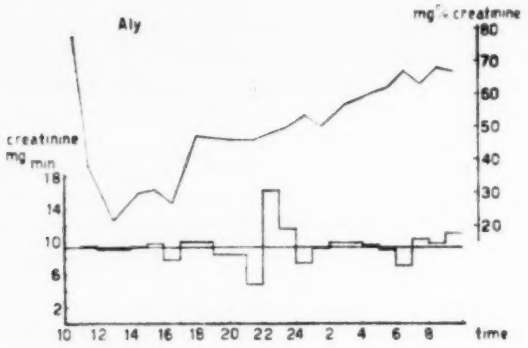

FIG. 2. Creatinine concentration and creatinine output per minute, plotted against time, of cow Aly, after drinking 85 l. of water

The high water intake and the resulting high volume of the urine excreted were subsequently found to be due to kidney disease (chronic atrophic nephritis) which had led to an inability to concentrate the urine.

In Tables I and II the results of the other "catheter experiments" are summarised. From Table I it will be seen that the quantities of urine collected per hour from Betsy in the stable were much smaller than those from Aly and the concentration accordingly higher. This cow Betsy drank less (20 l. in the morning and 30 in the afternoon) and moreover she gave 6 kg. of milk, whereas Aly was a dry cow. When Betsy was on pasture, however, the quantity and concentration of the urine resembled those of Aly in the stable.

In spite of the large differences in concentration the quantities of creatinine excreted per minute by Betsy in the stable and on pasture were practically the same. Movement when on pasture did not appear to affect creatinine excretion. On the other hand creatine excretion was much higher on pasture.

Notwithstanding the shortcomings of the method and the great variation in the concentration of the urine it is clear that the creatinine excretion per minute seems to be much more constant than the excretion of creatine or of creatinine plus creatine.

With the assistance of the Institute for Biological and Chemical Research on Field Crops and Herbage at Wageningen it was possible to obtain similar data for two other cows. In this case urine was collected every two hours but no attempt was made to ensure complete emptying of the bladder. The results are summarised in Table II. It will be seen that the standard error of the average creatinine excretion is somewhat higher than it was in Table I.

The effect of administration of KCl on the quantity and the concentration of the urine can be seen from the figures of Table II. As the animals had free access to drinking-water it may be assumed that the cow getting KCl drank more than the other.

The results of our second experiment in which a urinal was used for collection of urine are summarised in Table III. These figures, when compared with

TABLE I. URINE SAMPLES COLLECTED WITH A CATHETER. ESTIMATIONS EVERY HOUR

cow				urine per 60 min.			creatinine			creatine			creatinine + creatine		
name	live weight (kg.)	date	ration	average (ml.)	stand. error	stand. dev.	variation concentr. (mg. %)	average output per min. (mg.)	stand. error	stand. dev.	variation concentr. (mg. %)	average output per min. (mg.)	stand. error	stand. dev.	variation concentr. (mg. %)	average output per min. (mg.)	stand. error	stand. dev.
Ab	550	24–25 June 1959	water and hay *ad lib.*	1,286·5	118·0	528·0	22–78	0·47	0·48	2·14	14–48	6·11	0·33	1·46	36–119	15·60	0·85	3·82
Betsy	635	8–9 July 1959	water *ad lib.* 5 kg. hay 2½ kg. concentr.	386·0	23·5	117·5	97–300	11·24	0·54	2·68	28–114	3·82	0·25	1·26	118–384	14·99	1·79	8·97
Betsy	600	26–27 Aug. 1959	on pasture	1,340·5	177·96	795·5	14–68	10·31	0·17	0·76	20–64	11·265	0·42	1·88	30–134	21·50	0·53	2·37

TABLE II. URINE SAMPLES COLLECTED WITH A CATHETER. ESTIMATIONS EVERY TWO HOURS

cow				urine per 120 min.			creatinine				creatine			
No.	live weight in kg.	date	ration	average (ml.)	stand. error	stand. dev.	variation concentr. mg. %	average output per min. in mg.	stand. error	stand. dev.	variation concentr. mg. %	average output per min. (mg.)	stand. error	stand. dev.
II	584	14–15 Oct. 1959	grass *ad lib.* in stable + KCl	1,778·1	187·4	684·5	58–117	10·87	0·86	2·97	31–51	5·61	0·55	1·91
III	612	14–15 Oct. 1959	grass *ad lib.* in stable	1,031·6	110·7	383·0	84–171	9·51	0·90	3·12	54–113	6·29	0·70	2·43

TABLE III. URINE SAMPLES COLLECTED WITH URINAL

cow					creatinine				creatine				creatinine + creatine			
name	live weight in kg.	date	ration	number of samples	variation concentr. mg. %	average output per min. (mg.)	stand. error	stand. dev.	variation concentr. mg. %	average output per min. (mg.)	stand. error	stand. dev.	variation concentr. mg. %	average output per min. (mg.)	stand. error	stand. dev.
Aly	520	4–14 Dec. 1959	10 kg. hay, water *ad lib.*	42	42–96	9·48	0·11	0·73	10–25	3·22	0·10	0·67	56–79	12·66	0·16	1·04
Zwartschoft 4	590 pregnant 7 months	14–23 Dec. 1959	10 kg. hay, water *ad lib.*	30	31–107	8·57	0·19	1·03	27–165	9·71	0·45	2·46	53–272	18·32	0·53	2·89
Jansje	660 pregnant 7 months	11–15 Jan. 1960	hay and water *ad lib.*	17	80–154	10·66	0·28	1·16	38–88	5·83	0·31	1·29	120–226	16·64	0·52	2·15
Alie	600	21 Jan. 4 Feb. 1960	hay *ad lib.* 20 l. water	33	106–188	10·28	0·12	0·69	3–54	1·35	0·14	0·83	113–220	11·67	0·19	1·09
Kee*	670	21 Jan. 6 Feb. 1960	hay *ad lib.* 20 l. water	42	92–166	10·57	0·15	0·96	12–117	4·61	0·24	1·54	137–285	15·19	0·25	1·61
Aly	590	10–18 March 1960	hay *ad lib.***	28	48–90	11·41	0·15	0·81	4–19	1·99	0·17	0·90	56–102	13·40	0·23	1·21
Klaasje	568 pregnant 7 months	19 Jan.–2 Feb. 1960	6 kg. hay o·86 kg. concentr.	33	29–138	8·79	0·11	0·64	17–118	6·27	0·31	1·79	46–232	15·04	0·42	2·43

* This cow developed mastitis with fever during the experiment. In this short period the creatinine output rose from about 10·5 to about 12·5 mg. per minute and the creatine excretion diminished first from about 5 to about 1 mg. per minute and then rose to about 7·5 mg. per minute. This increase in creatinine excretion brought about by the rise in body temperature does agree with that calculated, using the figures of Borsook & Dubnoff (1947).

** This cow (with chronic nephritis) was deprived of water for some days to see whether the concentration of the urine would be increased. She lost 35 kg. weight but the creatinine concentration did not exceed 90 mg. %.

TABLE IV. VARIATION OF CREATININE CONTENT OF URINE SAMPLES WITH DIFFERENT SPECIFIC GRAVITIES

	creatinine (mg./100ml.)									
sp. gr.	0–30	31–60	61–90	91–120	121–150	151–180	181–210	211–240	241–270	271–300
1·000–1·005										
1·006–1·010	6	4								
1·011–1·015	1	2								
1·016–1·020	2	44	14							
1·021–1·025		22	23							
1·026–1·030		11	10	12	3					
1·031–1·035		3	21	21	21	15				
1·036–1·040			5	7	13	14	1			
1·041–1·045					4		4	3	3	
1·046–1·050					1		2	1		
1·051–1·055										1

those of Tables I and II, show striking differences in the standard error and standard deviations of creatinine excretion. As these are much smaller in Table III it is clear that not only is this a better method for collection of urine but also that the creatinine excretion is in fact quite constant.

The creatinine excretion of the cow Aly in June 1959 (Table I), is almost identical with that in December 1959 (Table III). However in March 1960, after the cow had been fattened for 3 months and increased in live weight by about 75 kg., the creatinine excretion had become somewhat higher. Although it does not necessarily follow that the heaviest cow always has the highest creatinine excretion (pregnancy, fatness and filling of the intestine play a rôle), creatinine excretion does show a tendency to increase with increasing body weight (greater active muscle mass). The work of Brody, Proctor & Ashworth (1934) supports this.

Creatine excretion on the other hand is much less constant. We believe that the creatine output is largely dependent on the quantity and composition of the feed, but this is still under investigation.

DISCUSSION

A clear picture of the constancy of creatinine excretion is given by Figs. 3 and 4 which depict the creatinine and creatine excretion of two cows whose urine was collected with a urinal. These figures show that cows of about the same live weight (Klaasje 568 kg., Alke 600 kg.) have a similar creatinine output per minute, whereas the creatine excretions diverge considerably. It is clear that the creatinine content is a better measure of the concentration of the urine than is the creatine present.

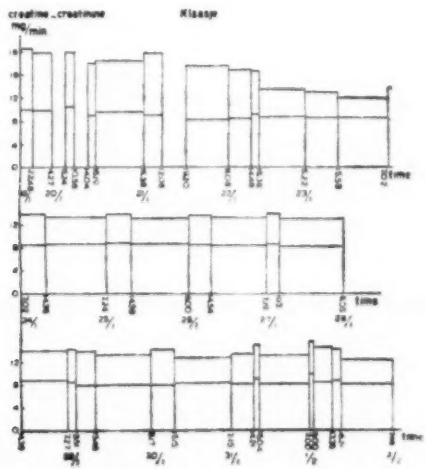

FIG. 3. Output of creatinine (lower line) and creatinine + creatine per minute (upper line) of cow Klaasje

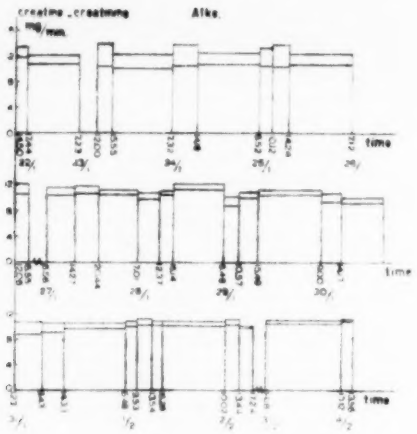

FIG. 4. Output of creatinine (lower line) and creatinine + creatine per minute (upper line) of cow Alke

In Figs 5 and 6 the excretion of creatinine and creatine in the urine collected by a catheter from the cow Betsy have been plotted. It can be seen that the variation in excretion was much greater than when the urine was collected with van Es & Vogt's apparatus (compare Figs. 3 and 4). On the other hand we see that the creatine excretion was much higher when the cow was on pasture.

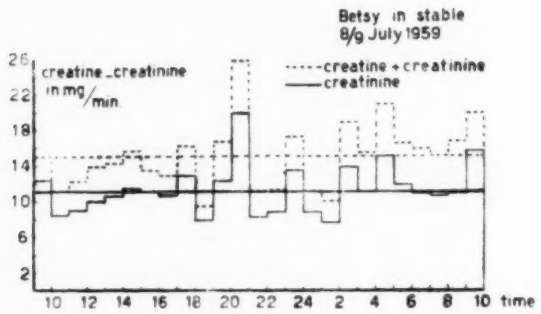

FIG 5

That the creatinine content is more reliable than the specific gravity as a measure of the concentration is quite clear from Table IV. It will be seen, for example, that when the specific gravity is between 1·031 and 1·035 the creatinine content may vary from about 45 to 165 mg. per cent, whereas with

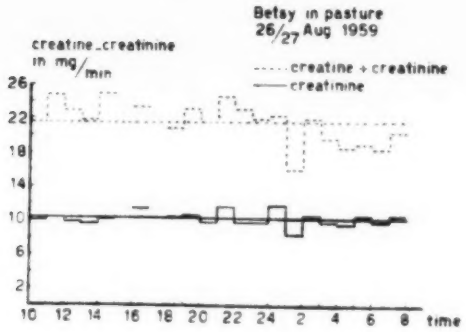

FIG 6

a creatinine content between 121 and 150 mg. per cent the specific gravity can vary from 1·028 to 1·048, although generally speaking a low specific gravity will correspond with a low creatinine content.

If the concentrations of urinary constituents are expressed as a quotient thus: $\frac{\text{content of constituent}}{\text{content of creatinine}}$ then the significance of determined constituents may be more reliably compared in different samples. A fortunate circumstance in this connection is that the estimation of the creatinine content is relatively simple.

As mature Dutch cows do not vary very much in live weight it is not considered necessary to make corrections for this factor. It may even be undesirable to do so as the heavier cows excrete somewhat more creatinine but they also have a greater metabolic capacity and so excrete a greater quantity of different metabolites.

SUMMARY

It has been demonstrated that just as with many other animals the cow also excretes creatinine at a constant rate. This appears to be true during day and night, notwithstanding the usually large creatine excretion or variations in water intake and subsequently in quantity of urine produced.

The creatinine content may therefore be used as a reliable measure of the concentration of the urine.

The quantity of creatine or creatine plus creatinine excreted is not constant.

It is proposed that the concentration of urinary constituents be expressed in terms of a creatinine quotient rather than in absolute units as in veterinary practice it is impossible to examine 24-hour urine samples.

ACKNOWLEDGEMENTS

We want to express our gratitude to Dr. M. Maguire of the Agricultural Institute, Dublin, for his helpful criticism in the preparation of the text of this

paper, and to the Institute of Biological and Chemical Research on Field Crops and Herbage at Wageningen for putting at our disposal a number of urine samples.

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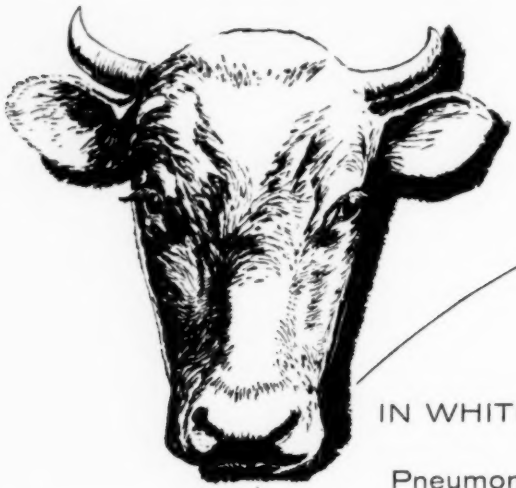

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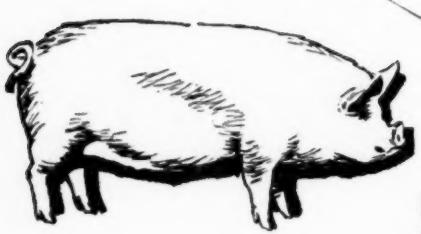

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OBSERVATIONS ON REPRODUCTION IN THE WILD RED FOX (*Vulpes vulpes*) AN ACCOUNT WITH SPECIAL REFERENCE TO THE OCCURRENCE OF FOX-DOG CROSSES

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According to the number of queries which have been received, veterinary surgeons are, from time to time, faced with questions concerning the possibility of fox-dog crosses. Sometimes they are asked to examine the results of such alleged crosses. Reports of suspected interbreeding of the wild red fox (*Vulpes vulpes*) and the domestic dog (*Canis familiaris*) occasionally appear in print. These communications are concerned, invariably, with bitches which have presented their owners with offspring bearing characteristics which may be considered fox-like. Rarely are sufficient facts presented to allow for an accurate assessment.

An extensive investigation on the reproductive processes of the British wild red fox (*Vulpes vulpes*) is being carried out in this division. From the data which have so far been collected and analysed certain facts have emerged which throw some light upon the problem of fox-dog crosses. It appears that the only study of the reproductive processes of foxes in Britain is that of Rowlands and Parkes (1935). The number of red foxes used in their investigation was very limited and the material for males consisted of seven immature animals and three adults. With one exception, all of these male animals had been maintained in captivity since an early age.

MATERIALS AND METHODS

In the present investigation, a preliminary report of which has appeared elsewhere (Creed, 1960), 137 adult male red foxes were obtained from twenty counties in England and Wales. These animals were shot in the field by gamekeepers, farmers or members of fox destruction societies. Although the carcasses were transported to the laboratory as quickly as was conveniently possible, the freshness of the material varied a good deal. The disadvantage of obtaining material which was not absolutely fresh was, however, offset by the fact that complete notes of the carcasses could be made in the laboratory. Moreover, while some of the material was not ideally suitable for precise

histological investigation, none of it was in such a condition that diagnosis of the stage of reproduction could not be made with certainty. From time to time, field excursions and attendance at fox shoots permitted material to be fixed immediately after the death of the animals.

Each carcass was weighed and certain measurements taken. The whole reproductive tract was then dissected out, weighed and measured. The testes and epididymides were removed separately from the tract and weighed, and representative pieces, together with other portions of the tract, were also prepared for histological examination. The fixatives used included 10 per cent formalin, Bouin's solution, Zenker-formol, Zenker-acetic, Susa and Gendre's fluids. Sections were stained with haematoxylin and eosin or with Masson's triple stain and Mallory's trichrome mixtures.

RESULTS

An examination of Fig. 1 shows that the weights of the testes of wild adult red foxes undergo changes which vary with the seasons. Each point on the chart represents the mean of the weights of the paired testes of all animals killed in a particular month during the years 1955, 1956, and 1957. It will be seen that the heaviest glands were obtained in December, January and

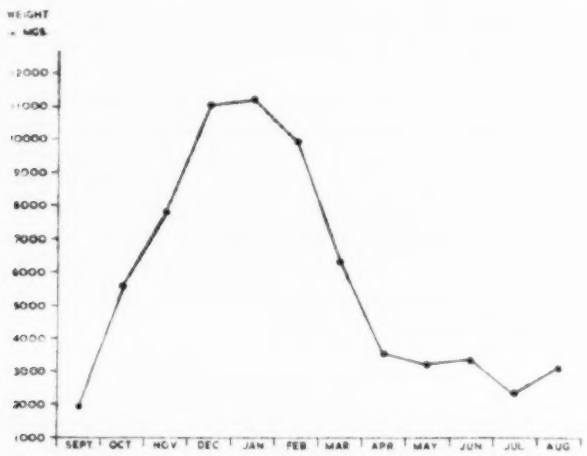

FIG. 1. Chart showing mean monthly weights of paired testes of foxes

February, with a peak occurring in January. Thereafter there was a rapid decline in the mean weight of the glands, the lowest weights being recorded between April and September. From October onwards mean weights increased progressively, to the peak in January.

Fig. 2, showing the mean weights of paired epididymides, plotted for the corresponding months of the same years, reveals a similar seasonal variation

in the weights of these organs, although the peak was attained in February instead of January and regression was not complete until May. This condition was maintained until approximately the end of September when testicular

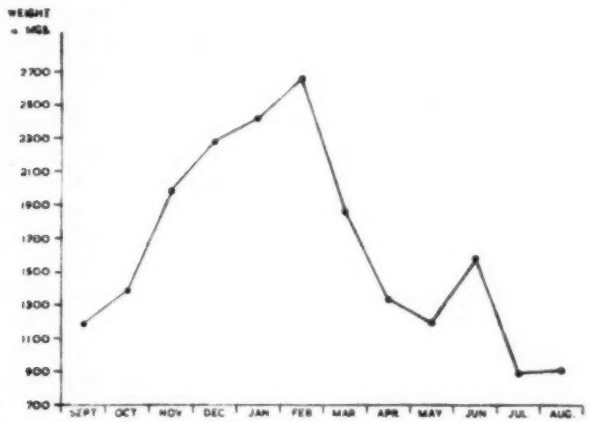

FIG. 2. Chart showing mean monthly weights of paired epididymides of foxes

resurgence began. The apparent secondary peak, occurring in June, is due to the inclusion of one animal with exceptionally heavy but inactive epididymides, in a month during which there were relatively few samples.

The seasonal variation in the weights of the testes and epididymides bears no relation to the absolute body weights in these animals. Histological examination of these organs, however, provides an explanation for this variation (Figs. 3–10).

The increase in the weights of testes, beginning in October, is primarily due to an increase in size of the seminiferous tubules. Specifically, the germinal epithelium begins to proliferate at this time, but, except in a small number of cases where spermatogenesis had proceeded as far as the production of a few spermatozoa, the majority of testes completely lack spermatozoa. Testes obtained in November all contain spermatozoa in the tubules but these are present in relatively small numbers. From December to February inclusive, spermatogenic activity is at its maximum and spermatozoa are present in large numbers in the tubules, the same being true for a few testes obtained in early March. In general, however, spermatozoa are relatively sparse in the majority of testes obtained in March and in early April. During the remainder of this latter month, full spermatogenic activity is in abeyance and spermatozoa are completely absent. This quiescent state of the seminiferous tubules is maintained throughout the summer months, May to September inclusive, during which time spermatogenesis does not, in general, proceed past the production of primary spermatocytes.

The increase in the weight of the epididymides is due to an enlargement of the ductus epididymis. This is brought about by proliferation of the pseudostratified epithelium, with a striking increase in the height of the individual columnar elements. The diameter of the ductus is also greatly increased and the lumen becomes progressively filled with spermatozoa. Thus, in October and throughout most of November, spermatozoa are absent from the ductus epididymis, whereas in late November they are present in relatively small numbers and from December to February they pack the ductus in masses. In only a few cases, obtained during early March, were large numbers of spermatozoa found in the ductus epididymis, and by April they were absent in the majority of animals and only very sparsely retained in the remainder. The testicular quiescence observed during the summer months (May to September) is reflected by a complete absence of spermatozoa from all the epididymides obtained during these months, including the exceptionally large ones of the animal killed in June and mentioned above. Accompanying the reduction in the numbers of spermatozoa during March and April there are retrogressive changes in the epithelium of the ductus epididymis, resulting in a diminution in its size. This reduced condition is maintained from May to September, the epithelium being of a stratified cuboidal or pseudostratified low columnar type.

Thus, the wild male red fox, unlike the dog or cat, undergoes seasonal gonadal changes. Reproductive activity is at its maximum during January and February, a time which coincides with ovulation in the vixen (Creed, 1960). Only from December to February are spermatozoa produced in numbers considered adequate for successful mating. In the majority of cases testicular rehabilitation has only just begun in November and by March retrogressive changes, following the period of maximum activity, are, in general, sufficiently far advanced to exclude the possibility of fertile coition.

DISCUSSION

The differing opinions concerning the possibility of fox-dog crosses are revealed in the following extract (Burton, 1948): "Relying on correspondence in 'The Field', I have stated in the August Journal (pp. 728–729) that unions between the English fox and domestic dog are fertile. In a letter to 'The Field' dated 20th November 1948 another correspondent remarks that the dog and fox are not of the same genus though they belong to the family Canidae and there is no authentic case of the European or any other fox crossing with the dog. The editor of 'The Field' confirms that the correspondent is correct and that it is impossible (despite all the rumours of dog-fox hybrids) for the two to interbreed".

The implication that a fox-dog cross is impossible because two different genera are involved is not in itself a valid objection to such an event. In the literature, several instances of intergeneric crosses are quoted in different groups of animals. Among the primates a cross between the crab-eating Macaque (*Macaca irus*) male and the Mandril (*Mandrillus sphinx*) female is reported. Among the Artiodactyla, crosses have occurred between the male

Zebu or Domestic Humped Ox (*Bos indicus*) male and the Gayal (*Bibos frontalis*) female on three occasions, and between the male *Bos indicus* and the female Yak (*Poephagus grunniens*). These cases of intergeneric crosses have occurred at the London Zoological Gardens (Zuckerman, 1953). Hybrids resulting from crosses between domestic yaks and domestic "yellow" cattle are commonplace in Tibet (Phillips, Tolstoy and Johnson, 1946). The male hybrids are sterile but the female ones are fertile. Other Artiodactyl crosses have also been reported (Gray, 1954), between the male Hog deer (*Axis porcinus*) and the female Fallow deer (*Dama dama*), and between the male Père David's deer (*Elaphurus davidianus*) and the female Red deer (*Cervus elephas*) by the Duke of Bedford (1940) and Hindle (1951). In this latter case, the fertility of the resulting hybrid is asserted. Among the Carnivora in the family Ursidae, Cook (1950) and Antonius (1951) have recorded crosses between the male Polar bear (*Thalarctos maritimus*) and the female Brown bear (*Ursus arctos*).

Thus in three mammalian orders, the Primates, the Artiodactyla and the Carnivora, intergeneric crosses have occurred. In all these cases, however, the crosses have taken place between animals in captivity and in some cases between animals which have reached a degree of domestication.

In considering those carnivores belonging to the family Canidae, the evidence of intergeneric crosses is of two types. The first type of evidence is supplied by the results, if any, of deliberately placing two captive animals of different sexes and genera together under controlled conditions and in isolation from others of their own kind. Results obtained by competent observers, under such conditions, are irrefutable. The conditions are, again, those of captivity. Fur breeders in North America have used such methods in attempts to introduce advantageous characteristics into their stocks. Cole and Shackelford (1947), in a review of fox hybrids, quote several well-substantiated instances of crosses between foxes of the genus Alopex (Arctic foxes) and those of the genus Vulpes based on evidence of this kind. One case quoted is that of the Russian worker Starkov (1940), who successfully artificially inseminated a silver fox (*Vulpes fulva*) with sperm from a "blue" arctic fox (*Alopex lagopus*), a single hybrid being produced. Thus the intergeneric cross Alopex × Vulpes is established under such conditions. The cross can occur in either direction but the resulting offspring are sterile, the chromosome complement of the parents differing considerably (Andres, 1938; Wipf and Shackelford, 1942).

The second type of evidence consists of observations on the morphological features and pelt characteristics, as well as the behaviour, of the suspected hybrid, usually a wild animal. Such evidence is often controversial and difficult to assess. Although their ranges overlap, there appear to be no records of the Alopex-Vulpes cross in the wild except for one instance in Iceland (Briem, 1941). In this case an escaped silver fox (*Vulpes fulva*) vixen was traced to its lair and was found to have mated with a "white" arctic fox (*Alopex lagopus*). Bezdek (1944), in the United States, has described the pelt obtained from an animal which he assumes to be the resulting hybrid of a cross in the wild between the red fox (*Vulpes vulpes*) and the grey fox (*Urocyon cinereoargenteus*).

An examination of the literature on alleged fox-dog crosses reveals little of

any positive value for the elucidation of the problem. Regarding the first type of evidence, namely that adduced from controlled matings, only negative results have been forthcoming. At different times the appropriate sexes of foxes and dogs have been penned together in isolation. Little or no sexual interest by either member of such a pair has been observed. In view of the fact that under conditions of confinement the wild red fox of Britain does not breed readily, this is not surprising. Clément (1923), after failing to breed from a fox-terrier bitch and a male fox (*Vulpes vulpes*), sacrificed the fox, removed its testes and made a suspension of spermatozoa in physiological saline. With this an attempt was made at artificial insemination. Subsequently, swelling of the mammary glands and the secretion of colostrum was exhibited but palpation revealed no foetus. Seventy-one days after artificial insemination, milk could be expressed from the teats and a week later the bitch had returned to normal. Clément suggests that the foetus may have died and was aborted. It seems more likely, however, that fertilisation did not occur and the manifestations were those of pseudo-pregnancy.

The second type of evidence, namely observations on morphological and pelt characteristics, and behaviour of suspected hybrids, has been presented on many occasions. Far too often cases have consisted of hearsay accounts and it has been impossible to trace the alleged hybrid to subject it to critical examination. In those instances where it has been possible to study such an animal, substantiation has not been forthcoming. A typical example of the latter case is that reported by Lloyd (1959) who investigated the result of an alleged cross between an Alsatian bitch and a male red fox. His examination of the product of this alleged mating, coupled with the fact that close questioning elicited changes in the original "story", led him to repudiate its authenticity. The fact that at the time of examination the "hybrid" was nurturing a litter of healthy puppies was additionally of significance. Photographs of the suspected hybrid suggest that Lloyd's conclusion was a correct one.

Throughout the literature concerned with fox-dog crosses which the writer has examined, rarely has a report mentioned the dates or month of the year at which the supposed crosses have taken place. In view of the findings in the present work, dates of events can be of the utmost importance. Practically all cases of suspected fox-dog crosses have been of the kind involving a bitch and a male fox. Invariably, the bitch concerned has "got out" during her season. At the turn of the century, bitches in oestrus were often staked out in the neighbourhood of a known fox covert in the hope that a subsequent cross might produce offspring with greater cunning and increased hunting ability. It seems clear that a proportion of the number of such cases reported can immediately be dismissed by an inquiry into the month of the year at which mating is supposed to have been effected.

The male red fox has here been shown to undergo seasonal changes in reproductive behaviour. From May to September inclusive, the testes are reduced in size and spermatogenic activity is in abeyance. Thus these animals are incapable of fertilising a vixen during this period. Regarding the possibility

of a fertile union between the bitch and the male fox during the remainder of the year, all that can be said is that such evidence as is available is far from convincing. On the other hand, such crosses remain a possibility during December, January and February when spermatogenic activity in the male fox is at its maximum. In view of the monogamous habit of the wild fox and the fact that the sex ratio in these animals reveals a preponderance of males over females, the possibility of the reciprocal cross occurring would seem to be even more remote. However, such a cross might never come to light.

In conclusion it may be said that although the fox (*Vulpes vulpes*) and the dog (*Canis familiaris*) belong to different genera, this is not, in itself, a sufficient reason for denying the possibility of a cross between them. It can, however, be stated with a high degree of certainty that during a restricted part of the year, from May to September, such crosses would be impossible. On the other hand, we cannot positively assert that they would not occur at other times although this seems unlikely and no authenticated cases have, as yet, been recorded.

SUMMARY

An investigation into the reproductive state of the male gonads in the British wild red fox (*Vulpes vulpes*) is reported. The male fox, like the vixen, is a seasonal animal and only during a certain limited time of the year can it produce spermatozoa. The problem of the alleged fox-dog cross is discussed in the light of this finding.

ACKNOWLEDGEMENTS

My most grateful thanks are due to Mr. H. V. Thompson, and members of his staff of the Ministry of Agriculture and Fisheries for kind co-operation in the obtaining of fox material. My thanks are also due to Mr. H. A. Burgess of this division for taking the photomicrographs, and for technical assistance.

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Explanation of Plates

Plate 1. Testes.

Fig. 3. Section of testis of fox killed on February 17th, showing size of seminiferous tubules. (Compare with Fig. 4). Bouin fixed. Masson. x 20.

Fig. 4. Section of testis of fox killed on August 13th, showing size of seminiferous tubules. Note considerable reduction in size as compared with Fig. 3. Bouin fixed. H. & E. x 20.

Fig. 5. Section of testis of fox killed on February 17th, showing the germinal epithelium of a seminiferous tubule. Spermatogenesis is proceeding fully and spermatozoa are present. Bouin fixed. Masson. x 635.

Fig. 6. Section of testis of fox killed on August 13th, showing the germinal epithelium of a seminiferous tubule. Spermatogenesis is limited and is not proceeding past the production of primary spermatocytes. Spermatozoa are absent. Bouin fixed. H. & E. x 635.

Plate 2. Epididymides.

Fig. 7. Section of epididymis of fox killed on February 17th, showing size of ductus epididymis. (Compare with Fig. 8). Bouin fixed. Masson. x 20.

Fig. 8. Section of epididymis of fox killed on August 13th, showing size of ductus epididymis. Note reduction in size as compared with Fig. 7. Bouin fixed. H. & E. x 20.

Fig. 9. Section of epididymis of fox killed on February 17th to show the nature of the epithelium of the ductus epididymis. Note the high columnar cells possessing stereocilia, and the presence of spermatozoa in the duct lumen. Bouin fixed. Masson. x 635.

Fig. 10. Section of epididymis of fox killed on August 13th to show the nature of the epithelium of the ductus epididymis. Note the low columnar or cuboidal cells lacking stereocilia, and the absence of spermatozoa in the duct lumen. Bouin fixed. H. & E. x 635.

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PLATE I

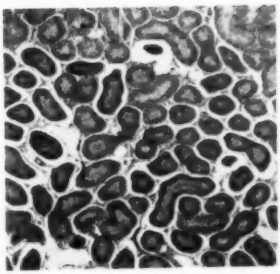
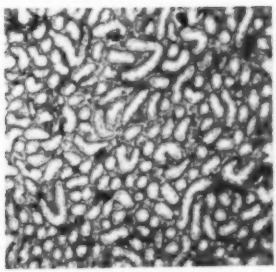


FIG. 3.



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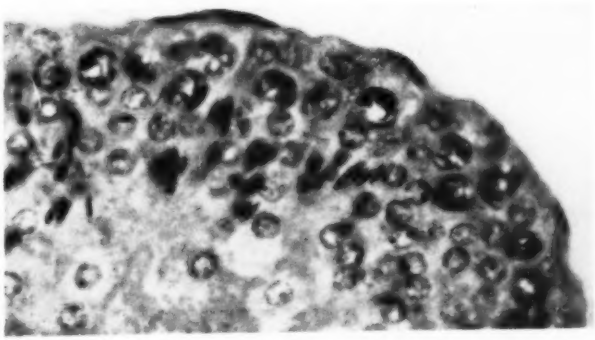

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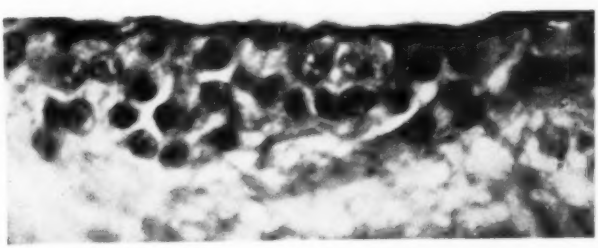

FIG. 6.

PLATE II

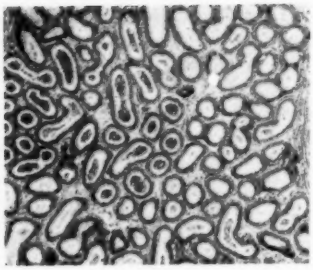

FIG. 7.

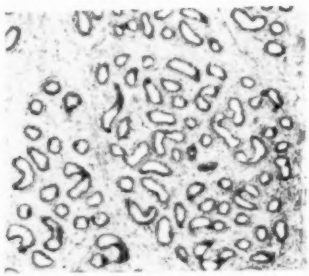

FIG. 8.

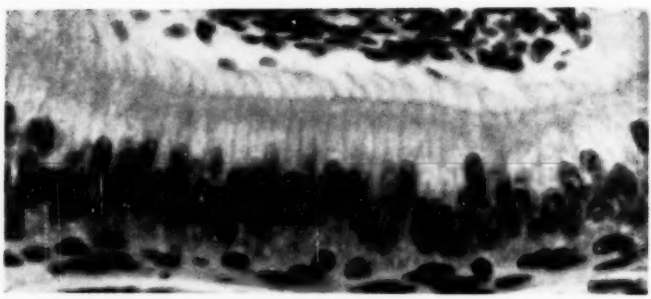

FIG. 9.

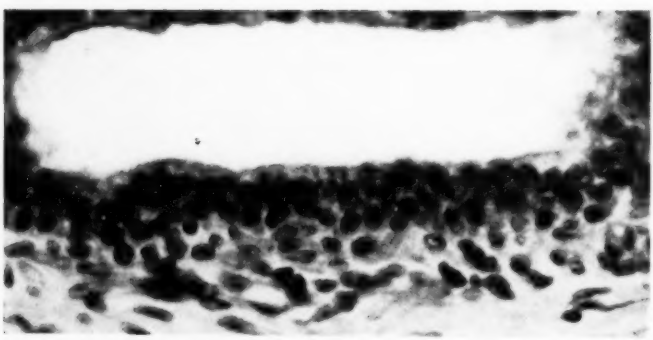

FIG. 10.

THE PRODUCTION AND PROPERTIES OF NEWCASTLE DISEASE VACCINE (KOMAROV STRAIN) IN NIGERIA

BY

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Newcastle disease was first reported on the West Coast of Africa in the Gambia (Hamilton, 1950), and later in the Gold Coast (Anon., 1951), the Southern Cameroons (Kirkby, 1951) and in Nigeria (Hill, Davis & Wilde, 1953).

At the fifth conference of the veterinary department in Nigeria in 1951, the high virulence of the disease in West Africa was reported and it was decided to protect all imported and all improved birds by use of a live vaccine. Vaccination was later extended to native birds in Nigeria.

In 1953 work began on the local preparation of a live vaccine because various transport difficulties were encountered in Nigeria when live vaccine was imported from South Africa. This imported vaccine was obtained from Onderstepoort Laboratories and consisted of glycerinated allantoic fluids containing the Komarov strain virus. Owing to conflicting reports elsewhere (Komarov & Goldsmit, 1946, 1947; Komarov, Goldsmit & Kahane, 1948; Zuydam, 1953; Ileri, 1956) it was decided to study the Komarov strain further despite the fact that the vaccine imported from South Africa had given good results in Nigeria.

MATERIAL AND METHODS

Three virus strains were used:

(i) *The Komarov strain obtained from the Onderstepoort Laboratories, South Africa.* This was a strain which had been isolated from a natural outbreak in Haifa, Israel and attenuated by intracerebral passage in chicks by Komarov & Goldsmit (1946).

(ii) *The Strain F virus which was obtained from the Ministry of Agriculture Laboratory, Weybridge.* This was of low virulence (Asplin, 1952).

(iii) *The Challenge virus which was isolated from a natural outbreak locally.* This was of high virulence. It produced symptoms and lesions typical of the acute disease and caused death within nine days. This virus was given to experimental birds by the intramuscular route because it permitted the inoculation of a fixed volume of virus dilution and because this

route had been shown by Doll, McCollum & Wallace (1951) to give a more severe challenge than either the oral or intranasal routes.

All virus strains were preserved by storing at −15°C. Fresh allantoic fluids were used at first but later the fluids were freeze-dried before storage. When resuspending dried materials, three ampoules were selected at random and their contents were pooled to give a representative sample.

Virus strains were cultivated by allantoic inoculation of ten-day-old embryonated eggs using an inoculum of 0·2 ml. for each egg. The eggs were then incubated for 48 hours at 37°C before harvesting. Fluids were collected only from eggs containing living embryos. The fluids from each egg were bottled separately and the contents of each bottle were spot-tested by the haemagglutination (HA) plate test. Only samples which were strongly positive were pooled and used for further work.

The HA plate test was carried out by placing four loops of a 5 per cent suspension of washed chick red cells on a white tile and mixing with one loop of the allantoic fluids to be tested. Strongly positive samples caused agglutination of the red cells in large clumps within 30 seconds.

The haemagglutination inhibition (HI) test was carried out by the method of Brandly and co-workers (1946) using doubling dilutions of antigen against a constant dilution of serum (1:10). Antigen was prepared from fresh Komarov strain allantoic fluids centrifuged at 4000 rev./min. to get rid of debris. Positive serum was prepared by inoculating birds, previously vaccinated, with challenge virus and bleeding the birds out three weeks later. Sera from birds showing HI titres greater than 1,280 units were pooled and stored at −15°C. Negative serum was obtained by bleeding out susceptible birds showing HI titres of 10 units or less. In the HI test 0.5 per cent washed chick red cells were used.

All materials in the HI test were distributed in 0·2 ml. amounts. The tests were left at room temperature (21° ± 2·2°C) and were read at 15-minute intervals for 45 minutes. The end-point was read as the last tube showing complete agglutination. In the interpretation of the results of the HI test sera showing titres of 40 HI units or less were regarded as negative.

All titrations of virus were carried out in tenfold dilutions using six eggs or six birds per dilution. Fifty per cent end-points were calculated by the method of Reed & Muench (1938).

The standard solution used for all dilutions was 0·85 per cent saline buffered with Sorensen M/15 phosphate buffer at pH 7·2.

Susceptible White Leghorn birds and eggs were obtained from the Department's own flock at Vom. Unless otherwise stated, three-month-old cockerels were used for experimental work.

SELECTION OF A VIRUS STRAIN

Preliminary work by Wilde (1953) showed that the effective dilution of vaccines prepared with the Komarov and F strains would lie between 2×10^{-2} and 10^{-3} dilutions of fresh allantoic fluids. This work was repeated using dried vaccine of both strains. The birds used were four-month-old cockerels and all

were challenged 21 days after vaccination. Both the vaccination and the challenge doses were 0·2 ml., given by intramuscular inoculation into the thigh. The challenge virus consisted of a 1:100 dilution of fluids which produced a titre of $10^7 LD_{50}$ in three-month-old birds.

Prevaccination HI tests showed that all birds had titres of less than 40 units. Table I summarises the results.

TABLE I

IMMUNISATION WITH KOMAROV AND F STRAINS

Strain	*No. of birds*	*Dilution*	*HI test post-vaccination range (units)*	*Deaths following challenge*
Komarov strain	3	10^{-3}	40–1,280	2/3
	7	2×10^{-3}	640–2,560	0/7
	3	10^{-3}	640–1,280	0/3
Strain F	4	10^{-3}	40– 320	4/4
	4	2×10^{-2}	640–1,280	0/4
—	4	10^{-2}	640–1,280	0/4
Controls	6	—	—	6/6

All birds which succumbed to challenge died or were killed *in extremis* between the 5th and 9th days after challenge. Symptoms and lesions were typical of the disease.

Table I shows that the Komarov and F strains appear to have given comparable results. This was, however, only in terms of serological response as, in all dilutions, strain F caused symptoms of diarrhoea, inappetence, nervous movements of the head and slight cyanosis of the combs within a week of vaccination. It was concluded that strain F was unsuitable for use as a vaccine and work with it was discontinued.

PREPARATION OF KOMAROV STRAIN VACCINE

A seed bank of the Komarov strain of virus was prepared and freeze-dried. This seed bank was shown to have a 50 per cent egg infective dose (EID_{50}) of $10^{8 \cdot 1}$.

For vaccine production, three ampoules of the seed batch were taken at random, the contents were resuspended with 0·5 ml. distilled water per ampoule and diluted to 1:1,000 with buffered saline containing 800 international units of penicillin and 2 mg. streptomycin per ml. Ten-day-old embryonated eggs were inoculated into the allantoic sac with 0·2 ml. of the dilution. The eggs were sealed with wax and incubated at 37°C for a further 48 hours. The allantoic fluid of each egg containing a living embryo was harvested and placed in a separate bottle. The contents of each bottle were HA plate tested and those showing strong positive reactions were pooled. The pool was mixed

with an equal volume of suspending fluid prior to desiccation. This fluid consisted of 75 per cent skim milk, 25 per cent M/15 phosphate buffer and 7 grams added lactose per 100 ml. The mixture was dispensed in 0·5 ml. amounts into sterile ampoules. Each ampoule contained 50 doses. This represents a dose of 0·05 ml. of the original allantoic fluid and at the dilution of 1:40 permits a fivefold safety factor. Desiccation was carried out in the primary system of an Edwards 30 PS centrifugal freeze-drier for 18 hours, following which the ampoules were transferred to the secondary drying system for a further 4 hours before being vacuum-sealed.

Titration of samples before and after desiccation showed the drop in titre to be at least 1 log.

Vaccine was tested for potency in susceptible three-month-old birds at dilutions of 10^{-1}, 10^{-2}, and 10^{-3}. Four birds were used for each dilution, 0·2 ml. of vaccine being given intramuscularly to each bird. Blood samples were collected from all birds immediately before vaccination and again fourteen days later.

A rise in antibody titres in the 10^{-1} and 10^{-2} groups to at least 640 HI units, together with smaller rises in the 10^{-3} group, was fixed as the minimum standard for the vaccine to pass the potency test.

METHOD OF ADMINISTRATION OF THE VACCINE

Administration of vaccine by stab puncture into the wing web as recommended for the imported vaccine was felt to present difficulties in inexperienced hands and a test was carried out to compare the efficiency of this method with the intramuscular inoculation of a fixed volume and dilution.

In the test one group of 21 birds was inoculated with 0·2 ml. of a 1:200 dilution of the vaccine intramuscularly in the thigh. A second group of 18 birds was given a 1:12 dilution of the same batch by stab puncture, dipping a 12 gauge needle into the vaccine and then making a stab in the wing web. Each bird received two stabs, the needle being dipped into the vaccine between stabs. Three weeks later both groups of birds and a group of 14 susceptible controls were given challenge virus ($10^{6 \cdot 1}EID_{50}$).

TABLE II

COMPARISON OF METHODS OF ADMINISTRATION OF VACCINE

	No. of birds	*Post-vacc. HI titre range (units)*	*Deaths following challenge*
Intramuscular inoculation	21	640–1,280	0/21
Stab puncture	18	80–1,280	5/18
Controls	14	—	14/14

The results in Table II show that all birds given the intramuscular inoculation resisted challenge whilst five of the 18 birds inoculated by stab puncture succumbed to challenge. These and the controls showed symptoms and lesions

typical of the disease. The post-vaccine HI titres were 160 units or lower in the birds which died. It was clear that the intramuscular method of inoculation of a fixed volume of vaccine gave better results.

DURATION OF VIABILITY OF THE VACCINE

After desiccation, vaccine is stored at −15°C before issue to the field. At this temperature the virus titre does not drop greatly over a period of 12 months as judged by egg titrations. Because of the varying conditions likely to be encountered after issue to the field, it is necessary to know the effect of storage at various temperatures and viability tests were therefore carried out.

The vaccine used in the tests was from a routine production batch. The predesiccation titre was $10^{9 \cdot 7}EID_{50}$ and the postdesiccation titre was $10^{8 \cdot 4}$ EID_{50}. Ampoules were stored, some at deep freeze temperature (−15°C), some at room temperature (21 ± 2·2°C), some in an incubator at 37°C, and some in a water bath at 45°C. Vaccine was titrated at weekly intervals. The results are shown in Fig. 1 where it will be seen that whilst there was an insignificant drop in titre at −15°C, the drop at room temperature was steady, averaging about half a log per week over eight weeks. The fall in titre at both water bath and incubator temperatures was so great that within two weeks the titre was below the minimum standard (10^6EID_{50}) set for issue.

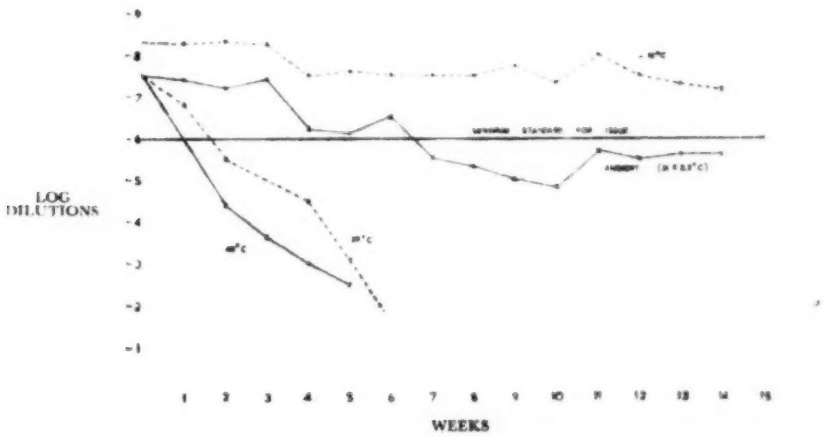

FIG. 1. Viability of Komarov strain Newcastle disease vaccine.

It is concluded that storage must be in a deep freeze (−15°C) if vaccine is to be kept for a considerable time, whilst for shorter periods the domestic refrigerator (4°C) would be suitable. For periods up to a week the vaccine can withstand temperatures likely to be encountered in the field without the titre falling below the standard.

DURATION OF IMMUNITY

Owing to the difficulty of re-inoculating flocks in the field a vaccine giving an immunity lasting more than a year was required. One vaccination, therefore, should be adequate to protect birds for life.

Fifty susceptible birds showing HI titres of not more than 10 units were vaccinated using a routine production batch of vaccine. Eighteen birds similarly tested were left as unvaccinated controls and were housed separately.

Post-vaccination HI tests were carried out 14 days after vaccination and then at monthly intervals until the birds were challenged.

The challenge virus was titrated in three-month-old susceptible birds and in eggs at the time of each challenge. Tenfold dilutions of the challenge virus were prepared and inoculated into six birds and six eggs per dilution.

TABLE III

DURATION OF IMMUNITY

No. of birds	*Immune state*	*Time of challenge (days post-vaccination)*	*Challenge virus titre LD_{50}*		*Deaths after challenge*
			Eggs	*Birds (3 mths.)*	
20	Vaccinated	308	$10^{6.6}$	$10^{8.3}$	0/20
9	Controls		,,	,,	9/9
10	Vaccinated	384	$10^{7.2}$	$10^{8.6}$	0/10
9	Controls		,,	,,	9/9

Twenty birds were selected for challenge 308 days after vaccination and a further 10 birds were challenged 384 days after vaccination. The results (Table III) showed that all the vaccinated birds were solidly immune to challenge doses which titrated at $10^{8.3}$ and $10^{8.6}LD_{50}$ in three-month-old cockerels and which killed the control birds. The second group of birds were challenged approximately 13 months after vaccination and withstood a challenge dose of $10^{8.6}LD_{50}$. From this degree of immunity it appeared that sufficient resistance to natural exposure would persist during a bird's productive life.

Table IV gives a summary of the HI antibody levels after vaccination.

Variations in the strength of the antigens from test to test imposed a ceiling on the serum titres and the highest titre obtained with the weakest antigen (>800) has been used in the calculation of the mean. All control birds showed titres of 40 units or less throughout.

Table IV shows that 30 days after vaccination all birds had developed high HI titres (>800) in response to vaccination. At 95 days the mean titre had dropped by almost 50 per cent to 460 units. From 95 days onwards there was a marked variation in the HI unit levels of individual birds. As all birds were

TABLE IV

HI ANTIBODY LEVELS AFTER VACCINATION (EXPRESSED AS HI UNITS)

Pre-vaccination		*Post-vaccination (in days)*				
		30	95	200	230	384
Mean	3	800	460	320	280	380
Range	0–10	800–6,400	160–3,200	20–1,600	20–640	160–640
S.D.	—	—	±140	±250	±250	±220
No. of birds *	47	47	47	37	37	8

* Three of the fifty birds were omitted from the calculation due to loss of serum samples.

solidly immune on challenge it was clear that the rate of decline of the HI titre and the level to which it fell bore little relation to the immune state of the bird.

VIRUS TRANSMISSION BY VACCINATED BIRDS

An experiment was carried out to find out whether vaccinated birds transmitted virus to susceptible in-contact birds and to what extent this occurred.

Ninety birds were vaccinated and confined in cement-floored pens together with 82 susceptible birds. HI tests were carried out on all birds immediately before and fourteen days after vaccination. Evidence of transmission was based on the rise in HI antibody titres of the in-contact birds.

TABLE V

VACCINE VIRUS TRANSMISSION

	No. of birds	*HI range (units)*		*Percentage birds showing titre rise*
		pre-vaccination	*post-vaccination*	
Vaccinated	90	0–20	640–2,560	100
In-contact	83	0–40	0–640	21·6

The results (Table V) show a definite rise in the HI titres in the in-contact birds. These ranged from 80 to 640 HI units, which was above the level accepted as a negative result (40 HI units). From this it was clear that transmission had occurred.

DISCUSSION

In view of the conflicting reports on the Komarov strain, it was compared with strain F, of low virulence, which was suggested as a possible vaccine strain by Asplin (1952). It was shown that strain F was still too virulent for use as a vaccine and it appeared to produce no higher degree of immunity than the Komarov strain on which work was then concentrated.

Komarov, Goldsmit & Kahane (1948) found that birds vaccinated with the Komarov strain resisted challenge nine months after vaccination. Komarov

and Goldsmit (1947) reported the paralysis of one bird out of 131 inoculated; there was no effect on egg production, and immunity developed within seven days. Zuydam (1953) reported that birds vaccinated with the same strain were partly immune on oral challenge three months later and that virus excretion occurred in the faeces of 15–20 per cent of vaccinated birds. Ileri (1956) found that the Komarov strain immunised solidly for twelve months and that a marked drop in egg production resulted from the vaccination of laying birds. Experience with the vaccination of laying flocks in Nigeria confirms this finding. Ileri (1956) found no evidence of post-vaccination paralysis but there have been field reports from Nigeria and Ghana which show that, following the vaccination of young birds in poor condition, a few cases of paralysis have resulted.

The use of a living virus vaccine involves the danger of a virulent mutation especially where contact infection occurs. This contact infection has been observed in young birds but no evidence of an alteration in the virus has been noted either in laboratory work or in the field. No symptomatic evidence of contact infection has been seen and all evidence was based on serological results. These findings agree with those of Haddow & Idnani (1946) who showed that "silent" transmission occurred following vaccination with the Muktesar strain.

It is realised that a live vaccine serves as a measure of prevention only. Before attempts at eradication can be made, a considerable rise in the standards of management and the ability to enforce the necessary sanitary measures are needed. Newcastle disease is under control where vaccination is carried out but repeated campaigns are required at regular intervals to maintain this position. The duration of immunity produced by the Komarov strain vaccine has enabled the field services to provide extensive cover in West Africa, where the disease is highly virulent.

The standard age for vaccination was selected arbitrarily as three months on grounds of convenience. This does not interfere with egg production and birds are large enough to withstand handling. In Nigeria it has been found possible to vaccinate safely birds as young as one week old but Brandly and co-workers (1946), Levine & Fabricant (1950) and Doll, McCollum & Wallace (1951) have shown that the passively transmitted antibody level in newly hatched chicks is sufficient to interfere with the development of active immunity. This passive immunity declines and disappears within 2–4 weeks. In Nigeria, it is recommended that, where vaccination of birds under one month old is necessary, they should be revaccinated at the age of three months. The results of viability tests showed that the vaccine can be maintained at deep-freeze temperature for more than three months. Viability remains above the standard for four weeks at room temperature and for one week at incubator temperature, permitting a reasonable margin of time for use where no cold storage facilities are available.

Field services in Nigeria have confirmed the value of the Komarov strain vaccine and this has been emphasised by the increasing demands for the vaccine from 20,000 doses in 1953 to 1,100,000 in 1959 (Anon., 1953–1959).

SUMMARY

Methods of preparation and administration of Komarov strain Newcastle disease vaccine are described.

The immunity conferred is of a high level, lasting more than one year, and the duration of viability is sufficient for its use under local conditions. This vaccine has proved suitable for the control of Newcastle disease in West Africa.

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NEWS AND NOTICES

UNIVERSITY OF GLASGOW

On 4th October, Sir Henry Dale, recently retired Chairman of the Wellcome Trust formally opened the Wellcome Laboratory, which has been erected with the aid of a grant from the Board of Trustees, at the University of Glasgow Veterinary Hospital. The facilities provided by the laboratory are quite unique and will allow the continuation and extension of programmes of research in the investigation of broad problems relating to medical science. The laboratory is being used by both medical and veterinary workers in the University of Glasgow.

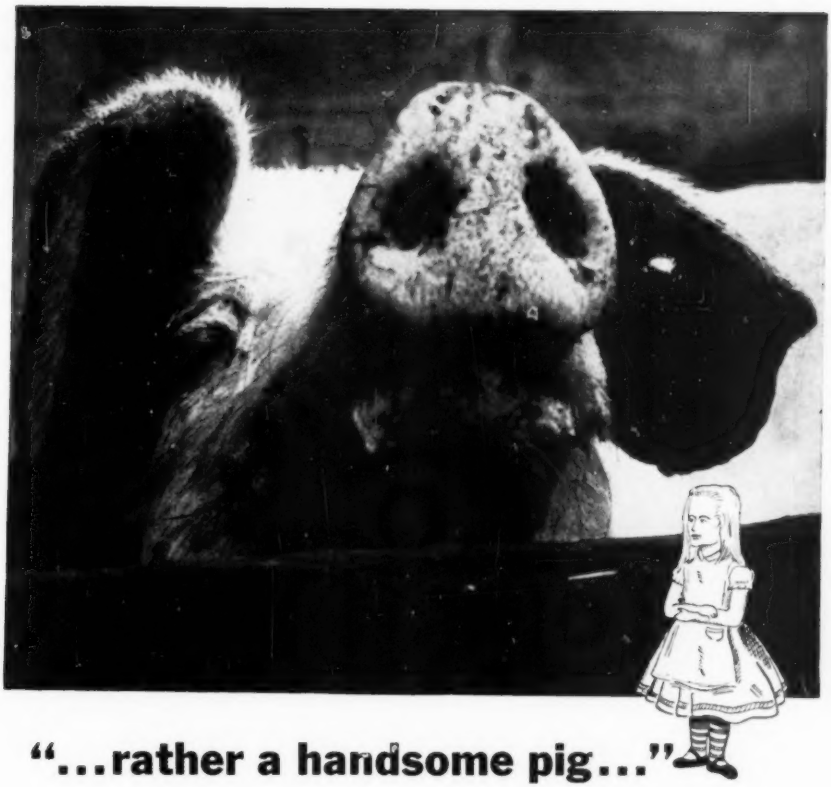
ROYAL COLLEGE OF VETERINARY SURGEONS

On 30th September the Council of the Royal College of Veterinary Surgeons held its last meeting at 9 & 10 Red Lion Square. The senior Vice-President, Mr. G. N. Gould, presided, and the quarterly meeting of the Council was followed by a special meeting, attended by past members and other guests, at which the long association of the profession with Red Lion Square was recalled.

The offices of the College will shortly be moved to temporary accommodation in Kensington while a permanent home is being sought.

ANIMAL HEALTH TRUST

At a luncheon held at the Hyde Park Hotel, London, on 17th October, Dr. W. R. Wooldridge, Scientific Director of the Animal Health Trust, gave an account of the Trust's progress. The Duke of Norfolk was in the chair and the Minister of Agriculture was the guest of honour.



"...rather a handsome pig..."

". . . so she set the little creature down and felt relieved to see it trot away quietly into the wood." "If it had grown up," she said to herself, "it would have made a dreadfully ugly child; but it makes rather a handsome pig, I think." And she began thinking over other children she knew, who might do well as pigs, and was just saying to herself, "If one only knew the right way to change them—"

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